Phosphate Sensor Experiment Protocol

We have done a characterization on P_{phoA} and P_{phoBR} using Luria broth (LB) medium. Quantitative characterization on the promoter was done by measuring the fluorescence signal intensity using an EnVision multilabel reader.

Filter used on EnVision Multilabel Reader:

- Absorbance: Photometric 595nm,
- Excitation: 485nm FITC,
- Emission: 535nm FITC,
- Mirror module: FITC (403) on top.

E. coli strain DH10B was used, and the concentration of the characterization of P_{phoA} was from 0 to 300 µM phosphate, with an intervals of 50 µM.

60 µl of antibiotics was added to each medium.

Preparing test medium with different concentration of phosphate

We have prepared a solution of M9 minimal medium (J. Sambrook & D.W. Russell, 2001) and a solution of M9 minimal medium with Tris replacing phosphate. Test medium with different concentration of phosphate (0, 10, 30, 50, 100, 150, 200, 250, 300 µM) were made by mixing the 2 solution in the following ratio.

Final Phosphate concentration (µM)	**M9 minimal medium (µl)**	**M9 minimal medium without phosphate (replaced by Tris) (ml)**
0	0.00	60
10	8.58	60
30	25.73	60
50	42.85	60
100	85.71	60
150	130.43	60
200	171.42	60
250	216.26	60
300	260.87	60

Characterization

Bacteria hosting pSB1C3-BBa_P_{phoA}-I13504 (or pSB1C3-BBa_P_{phoBR}-I13504), positive control and negative control were first grown overnight in 5 ml Luria broth (LB) medium containing chloramphenicol at 37°C. The bacteria were then washed twice with 3 ml M9 minimal medium without phosphate (replaced by Tris), containing ampicillin. Then, the cells were resuspended in 5 ml M9 minimal medium without phosphate (replaced by Tris) to obtain a final OD_{600} of 4.50 µl of the prepared cell suspension were then added into 950 µl of test medium with different concentrations of phosphate (containing chloramphenicol) in the 96-well deep well plate and further incubate at 37^{o}C until the OD_{600} of the cells reaches the mid-log phase. The fluorescence output were then measured using EnVision multilabel reader.

The results were obtained by biological triplicates and technical triplicates.

Reference

Sambrook, J., & Russell, D. W. (2001). Molecular cloning. A laboratory manual. Third. *Cold pring Harbor Laboratory Press, New York*.